

The Influence of Protein and Energy Density in Commercial Diets on Growth, Body Composition, and Nutrient Retention of Sunshine Bass, *Morone chrysops* ♀ × *Morone saxatilis* ♂, Reared at Extreme Temperature

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Abstract

Three growth trials were conducted with juvenile sunshine bass reared at temperatures typical of winter or summer pond culture in the Southeastern USA. The trials were designed to determine if there was an advantage to feeding a commercial high-protein/high-fat diet during winter and a low-protein/high-fat diet during summer. In the first trial, two commercially extruded, practical diets (40% protein/10% lipid vs. 48/18) were fed to apparent satiation to fish held in variable cool water (8–20 C) or constant 26 C water for 14 wk. Temperatures in the cool water (8–20 C) tanks were chosen to simulate winter–spring conditions. In the second and third trials, factorial experiments were conducted in which four commercial diets (35/10, 35/15, 40/10, and 40/15) were fed to apparent satiation to fish held at 29 or 32 C for 4 wk to simulate near optimal versus extreme summer water temperatures. Survival was 100% in the first trial, 99% or more in second trial, and 90% or more in third trial. At 8 C, minimal feeding (0.3% of body weight/day) was observed and fish lost weight. Fish consumed feed daily and gained weight at 10 C or above. At 8–20 C, intake of the 48/18 diet was less than that of the 40/10 diet only when water temperature was above 15 C; however, gain was not different. At 26 C, fish consumed less of the 48/18 diet for greater gain than fish consuming the 40/10 diet. At 8–20 C, feed efficiencies increased with temperature and diet protein/lipid level. Visceral and whole-body fat tended to be diet-dependent but not temperature-dependent and averaged 4% higher in fish fed the 48/18 diet. Muscle ratio and whole-body protein retention were temperature-dependent but not diet-dependent. Energy retention was positively related to both temperature and diet nutrient density. At 29 and 32 C (summer culture trials), daily gain and final fish weight were positively related to protein but not lipid level in the diet. At 29 C, fish consumed less 35% protein diet than 40% protein diet regardless of dietary fat level, whereas consumption did not differ among diets at 32 C. Feed efficiencies were positively related to both dietary protein and lipid level at 29 and 32 C. The effects of diet nutrient density on fat versus muscle content and energy and protein retention differed at 29 and 32 C. Intraperitoneal fat (IPF) appeared unaffected by diet at 29 C, where muscle ratio was higher at the higher protein level (40%). At 32 C, IPF was positively related to dietary protein and fat, where muscle ratio was unaffected by diet. At 29 C, both energy and protein retention appeared unrelated to diet, whereas at 32 C energy retention was positively related to dietary fat level and protein retention was positively related to both protein and fat levels in the diet. In all trials, liver size (hepatosomatic index) was a sensitive indicator of culture temperature and dietary protein and fat levels. Livers from fish held in cool water were larger than those from fish held at 26 C, and fish fed diets of lower nutrient density had larger livers than fish fed diets of higher nutrient density, regardless of culture temperature. Hybrid striped bass showed remarkable adaptation to extremely high culture temperature and results suggest that judicious feeding of nutrient-dense diets when temperatures are above 15 C will improve production efficiency.

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The production cycle of sunshine hybrid striped bass, *Morone chrysops* ♀ × *Morone saxatilis* ♂, in earthen ponds in the Southeastern USA usually extends from 14 to 18 mo (Hodson and Hayes 1989; D'Abramo and Frinsko 2008). Although optimum rearing temperatures are 25–27 °C for hybrid striped bass (Hodson 1989), producers typically stock fingerlings from June to September that not only require over-wintering in ponds, but also require feeding during the hot months of the following summer in order to achieve marketable size by the end of the second fall (D'Abramo and Frinsko 2008). During the winter, pond temperatures often fall below 15 °C, where feeding activity in warm water species is attenuated. Sunshine bass are hybrids of temperate zone species; however, anecdotal reports suggest that feeding activity continues at lower temperatures than those reported for species such as catfish or tilapia. Additionally, episodes of warmer weather occur during winters in the southeast USA and producers often feed during those times to maintain fish health, stem winter weight loss, or improve growth during milder temperatures. In another scenario, producers may stockpile juvenile fish in cool water (<15 °C) facilities in order to ensure a constant supply of fingerlings for year-round production. In either case, there can be great losses because of predation, disease, cannibalism, and poor water quality associated with reduced feeding activity and decomposition of uneaten feed. The current practice is to cease feeding sunshine bass when temperatures fall below 16 °C (D'Abramo and Frinsko 2008), or to reduce feeding frequency to once per week (Hodson and Hayes 1989).

During the summer months, on the other hand, temperatures in southern ponds often exceed those considered optimum for rearing *Morone* hybrids (Hodson 1989; D'Abramo and Frinsko 2008). Physiological data confirm that evacuation rate, nutrient assimilation, and metabolism are Q10 temperature-dependent in fish (Jobling et al. 1977; Santulli et al. 1993). A reasonable hypothesis is that nutrient assimilation in summer might be less efficient in a temperate bass like *Morone* at a time when energy needs are greatest (Hidalgo et al. 1987;

Jobling 1995). In this case, greater excretion of dietary nitrogen would be expected that in turn could degrade water quality during the most stressful period of production (Hidalgo and Alliot 1988).

Previous studies suggest that the optimum dietary protein level is 40% of dry diet (Brown et al. 1992) and the optimum energy/protein (E/P) ratio is 8 kcal/g protein at 24–27 °C (Nematipour et al. 1992) in sunshine bass. Kelly and Kohler (1999) found that cold tolerance of several *Morone* spp. was influenced by dietary-induced muscle fatty acid composition. Woiwode and Adelman (1991) reported feed consumption of a diet composed of 29% protein and 7% fat increased as water temperature increased from 6.5 to 29.2 °C, then decreased as temperature increased to a maximum of 33.1 °C in palmetto bass, *M. saxatilis* ♀ × *M. chrysops* ♂, fed to satiation. Stone and Sidell (1981) investigated the relative *in vitro* hepatic utilization of radiolabeled carbohydrate versus lipid in striped bass reared at temperatures 5 to 25 °C and found significant temperature dependence in nutrient substrate utilization. Burr et al. (2006) compared performance and nutrient utilization in hybrid striped bass with that of red drum fed semi-practical diets containing ultra high protein and fat levels and found significant diet × species interactions. Finally, Keembiyehetty and Wilson (1998) evaluated dietary energy/protein ratio in semi-purified diets at water temperatures assumed to be optimum for growth (27 °C) or often observed in pond culture in the Southern USA (32 °C) and found significant effects of culture temperature on growth, body composition, and nutrient utilization in sunshine bass.

Therefore, the current research was designed to evaluate dietary protein and energy manipulation over the entire range of relevant culture temperatures for hybrid striped bass using diets that are commercially applicable and was designed to address producer inquiries as to whether there are advantages to (1) offering a nutrient-dense, perhaps more expensive, diet during cooler temperatures and (2) offering a high-energy, low-protein diet during summer temperatures. Another difference between this

study and previous work is that the test diets were formulated and manufactured to typical commercial composition and consistency and represented potentially marketable products.

Materials and Methods

Winter Culture Trial

In the first trial, two commercially formulated, floating diets were extruded from practical ingredients and were designated 40/10 or 48/18 to signify the % protein/% lipid ratio on an as-is basis (Table 1). These proprietary formulations were commercially available for use in the hybrid striped bass industry and were formulated to meet or exceed all known nutritional requirements of hybrid striped bass (Cargill™ Animal Nutrition/Burris Mill & Feeds, Inc., pers. comm.). The diets were fed twice daily to apparent satiation to fish held in 600-L round, fiberglass tanks at 8–20 C or 26 C for 14 wk. Juvenile sunshine bass, *M. chrysops* × *M. saxatilis*, obtained from a commercial hatchery (Keo Fish Farms,

Keo, AR, USA) were reared indoors at the USDA/ARS – H. K. Dupree Stuttgart National Aquaculture Center, Stuttgart, Arkansas, USA, on a commercial hybrid striped bass diet (Cargill™ Animal Nutrition/ Burris Mill & Feeds, Inc., Franklinton, LA, USA). Subsequently, the fish were acclimated to their initial temperatures (8 C vs. 26 C) for 2 wk prior to the beginning of the experiment. Each of eight tanks was stocked with 40 randomly selected fish which averaged 154 ± 12 g (\pm SD) each. Four of the tanks (cool water) were connected to a system providing flow-through well water that was mechanically chilled and four of the tanks (control) were connected to a system providing flow-through well water at a constant 26 C. Each diet was randomly assigned to pairs of tanks held at each temperature ($N = 2/\text{diet} \times \text{temperature treatment}$; 4 tanks/diet). Water was provided at a rate of 12 L/min to all tanks and ample aeration was provided via air stones connected to a mechanical blower. In a strategy similar to that employed by Buentello et al. (2000), temperatures in the cool water

TABLE 1. Composition of commercial hybrid striped bass diets with different energy : protein ratios fed to juvenile sunshine bass at temperatures simulating winter and summer culture in the Southeastern USA.

	Diet designation ^a				
	35/10	35/15	40/10	40/15	48/18
Formulated composition (as-fed)					
Animal protein (%) ^b	63	64	63	63	75
Vegetable protein (%) ^b	37	36	37	37	25
Protein (%)	35.1	35.0	40.0	40.0	48.0
Fat (%)	10.0	15.0	10.0	15.0	18.0
Fiber (%)	4.5	4.1	4.7	3.7	2.0
Ash (%)	8.7	8.6	9.3	8.9	10.0
Moisture (%)	10.0	10.0	10.0	10.0	10.0
NFE carbohydrates (%) ^c	41.7	37.3	36.0	32.4	22.0
Energy (kcal/g) ^d	3.97	4.24	3.94	4.25	4.42
E:P ratio (kcal/g)	11.3	12.1	9.9	10.6	9.2
Analyzed composition (dry-weight basis)					
Protein (%)	38.0	39.3	43.5	43.9	53.1
Fat (%)	11.1	16.6	11.1	16.4	20.3
Moisture (%)	8.4	8.6	8.5	8.1	7.7
Gross energy (kcal/g)	4.78	4.82	4.76	4.94	5.44

^aDiets are designated % protein/% lipid on an as-fed basis and were obtained from Cargill™ Animal Nutrition/Burris Mill & Feeds, Inc., Franklinton, Louisiana, USA.

^bPercent of total protein provided by this component.

^cNFE carbohydrate = $100 - (\% \text{ protein} + \% \text{ fat} + \% \text{ fiber} + \% \text{ ash})$.

^dEstimated energy = $(4 \text{ kcal} \times \text{g NFE}) + (4 \text{ kcal} \times \text{g protein}) + (9 \text{ kcal} \times \text{g fat})$.

(8–20 C) tanks were chosen to simulate winter–spring conditions in the Southeastern USA. Fish were fed their respective diets for 2 wk before the temperature in the cool water group was increased to 10 C due to a lack of feeding response at 8 C. Thereafter, fish in the cool water group were held at 10, 15, and 20 C for 4 wk each during which feeding continued.

Summer Culture Trials

In the second and third trials, four commercially formulated, floating diets were extruded from practical ingredients and fed in a 2 (protein levels) \times 2 (lipid levels) factorial design. The four diets were designated 35/10, 35/15, 40/10, or 40/15 to signify the % protein/% lipid ratio on an as-is basis (Table 1). These proprietary formulations were commercially available for use in the hybrid striped bass industry and were formulated to contain the same ratio of animal to vegetable protein and to meet or exceed all known nutritional requirements of hybrid striped bass (Cargill™ Animal Nutrition/ Burris Mill & Feeds, Inc, pers. comm.). The summer culture trials were conducted in the same tanks and manner as described above; however four additional tanks were added to the system. Because of space limitations, two trials were conducted in series with different batches of fish in each trial. The first trial was conducted at 29 C for 4 wk immediately followed by the second trial conducted at 32 C for 4 wk. These temperatures were chosen to simulate consistent versus extreme summer pond temperatures in the Southeastern USA. Each of the 12 tanks was stocked with 20 randomly selected juvenile sunshine bass for a total of 240 fish at each temperature setting. Fish stocked in the 29 C trial averaged 103 ± 9 g each, and fish stocked in the 32 C trial averaged 113 ± 11 g each. Fish were acclimated to their initial temperatures for 2 wk prior to the beginning of each trial. Each diet was randomly assigned to three tanks of fish ($N = 3/\text{dietary protein} \times \text{lipid treatment}$).

At each feeding, fish were deemed satiated when about 2 g (10–12 pellets) of uneaten feed was left in each tank for at least 15 min from the last addition of feed. Water temperature was

recorded and feed consumption was quantified and recorded after each feeding by differential weighing of individual feed buckets assigned to each tank of fish. Fish in each tank were collectively weighed at stocking and every 2 wk thereafter in batches of five fish each. Daily feed intake in each tank of fish was expressed as a percent of initial body weight consumed per day according to the following relationship:

Daily feed intake

$$= ([\text{total feed consumed [g as-is]}] / [\text{initial fish weight [g]}] \times 100) / t$$

where t = time in days.

Average daily gain (ADG) was expressed as the percent change in initial body weight per day according to the following relationship:

$$\text{ADG} = ([W_f - W_i] / W_i \times 100) / t$$

where W_f = final weight of fish in the tank (g) and W_i = initial weight of fish in the tank (g).

Fish Sampling and Compositional Analyses

At the end of the trials, fish in each tank were individually weighed and counted. Five fish per tank were randomly selected, euthanized, and frozen for the later determination of body compositional measures, defined as follows:

Hepatosomatic index (HSI)

$$= \text{liver mass} \times 100 / \text{fish mass};$$

Intraperitoneal fat (IPF) ratio

$$= \text{intraperitoneal fat mass} \times 100 / \text{fish mass};$$

Muscle ratio (MR)

$$= \text{fillet yield with ribs} \times 100 / \text{fish mass}.$$

An additional random sample of five fish per tank was collected and frozen for the later determination of proximate composition according to standard methods (AOAC 2000). Frozen fish were sectioned and passed through an industrial meat grinder. Ground sections were pooled for each fish and thoroughly mixed. This process was repeated two additional times

prior to aliquots being taken for analysis. Briefly, protein ($N \times 6.25$) was determined by the Dumas method using a LECO nitrogen analyzer (FP428, LECO Corporation, St. Joseph, MI, USA). Total energy was determined by isoperibol bomb calorimetry (Parr1281, Parr Instrument Company Inc., Moline, IL, USA). Whole-body lipid was performed by gravimetric quantification following chloroform : methanol extraction (Folch et al. 1957). Protein and energy retention efficiencies were calculated according to the following relationships:

$$\text{Protein retention efficiency (PRE)} \\ = \text{protein gain} \times 100/\text{protein fed};$$

$$\text{Energy retention efficiency (ERE)} \\ = \text{energy gain} \times 100/\text{energy fed}.$$

Statistical Analysis

The SAS software program PROC Mixed (Software Release 9.1, 2002-2003, SAS Institute, Inc., Cary, NC, USA) was used to conduct factorial, mixed model analyses of variance of

response to diet and temperature (main effects) in the winter culture trials, and dietary protein (35 vs. 40%) and lipid (10 vs. 15%) in each of the summer culture trials (29 and 32 C). Fish tank within the relevant main factor combination (diet \times temperature or protein \times lipid) was considered a random effect with uncorrelated (compound-symmetric) variance-covariance structure. Differences among mean responses were separated using the Tukey-Kramer method for pair-wise comparisons (Prins et al. 2003). Treatment effects were considered significant at $P < 0.10$.

Results

Winter Culture

Feeding and Growth. When 14-wk data are compared, survival was 100% and daily intake of the 48/18 diet was less than that of the 40/10 diet regardless of culture temperature. However, examination of intake within temperature periods reveals that intake only differed between diets when water temperature

TABLE 2. Daily feed intake (DFI) of juvenile sunshine bass (154 g) fed two commercial diets differing in protein and energy ratio and reared for 14 wk at temperatures simulating winter-spring in the Southeastern USA. Cool refers to tanks in which temperatures were warmed from 8 to 20 C; warm tanks were held at 26 C. Values in each column are mean response of duplicate tanks of fish in each diet \times temperature treatment combination for 2-wk (8 vs. 26 C), 4-wk (10, 15, or 20 vs. 26 C) or 14-wk (cool vs. warm) intervals.

Diet ^b	C	DFI ^a	C	DFI	C	DFI	C	DFI	C	DFI
40/10	8	0.29	10	0.52	15	1.09x	20	2.01	Cool	1.22
	26	3.31	26	2.34	26	2.26z	26	1.78	Warm	3.96
48/18	8	0.29	10	0.49	15	0.98x	20	1.68	Cool	1.07
	26	3.24	26	2.12	26	1.75y	26	1.19	Warm	3.56
Pooled SEM		0.08		0.06		0.07		0.07		0.12
Main effect means ^c										
Diet	40/10	1.80		1.43		1.68		1.89z		2.71z
	48/18	1.76		1.31		1.36		1.44y		2.43y
Temp	Cool	0.29Y		0.51Y		1.03		1.84Z		1.21Y
	Warm	3.27Z		2.23Z		2.01		1.48Y		3.93Z
Analysis of variance, Pr > F										
Diet (D)		0.67		0.15		0.02		0.01		0.10
Temperature (T)		<0.001		<0.001		<0.001		0.02		<0.001
D \times T		0.67		0.24		0.07		0.18		0.32

^aAmount of feed consumed/100 g of initial fish in that temperature interval per day.

^bDiets are designated % protein/% lipid on an as-fed basis.

^cMain effect (least squares) means in each column with different letters (lowercase = diet differences; uppercase = temperature differences) are different ($P < 0.10$) as determined by the Tukey-Kramer method for pair-wise comparisons (Prins et al. 2003). In the case of significant interaction, diet \times temperature treatment means with different letters are significantly different.

was above 15 C. For hybrid striped bass in this size range (about 150–600 g), feed intake in the cool temperature tanks averaged less than 1.5% when intake at 26 C was more than 3% of body weight. Daily feed intake in fish held at 26 C was 3.3% of initial body weight per day and subsequently decreased to an average 1.5% as fish grew from a mean weight of 154–590 g over the course of the 14-wk trial (Table 2). On the other hand, feed intake was less than 0.3% and fish lost weight when held at 8 C for 2 wk. At 10 C, feed intake averaged 0.5% and fish grew from 154 g to about 163 g in 4 wk. At 15 C, feed intake increased to an average 1.0% and fish grew from 163 g to about 185 g in 4 wk. At 20 C, feed intake averaged 1.8% and fish grew from 185 g to about 243 g in 4 wk.

When 14-wk data are compared, there were no differences in ADG because of diet when water temperature was less than 26 C (Table 3). ADG of fish held in cool water was about 0.6% of body weight over the 14-wk trial, whereas

ADG of fish fed the 48/18 diet (3.1%) was significantly greater than that of fish fed the 40/10 diet (2.6%) at 26 C. Within each temperature period, ADG of fish fed the 48/18 diet was slightly higher during the time fish were held at 10 C.

Feed efficiency (FE) was significantly and independently related to both diet and culture temperature (Table 4). Fish fed the 48/18 diet exhibited greater FE than fish fed the 40/10 diet, and fish held at 26 C exhibited greater FE than fish held in cool water (Table 4). Within each temperature period, FE was not significantly different between diets until water temperature was above 15 C.

Body Compositional Indices and Retention Efficiencies

Liver size, as measured by HSI, ranged from 1.7 to 2.7% of body weight and was influenced by diet, depending on the culture temperature (Table 5). Livers from fish held in cool water were larger than those from fish held at 26 C, but diet did not influence liver size at 26 C.

TABLE 3. Average daily gain (ADG) of juvenile sunshine bass (154 g) fed two commercial diets differing in protein and energy ratio and reared for 14 wk at temperatures simulating winter–spring in the Southeastern USA. Cool refers to tanks in which temperatures were warmed from 8 to 20 C; warm tanks were held at 26 C. Values in each column are mean response of duplicate tanks of fish in each diet × temperature treatment combination for 2-wk (8 vs. 26 C), 4-wk (10, 15, or 20 vs. 26 C) or 14-wk (cool vs. warm) intervals.

Diet ^b	C	ADG ^a	C	ADG	C	ADG	C	ADG	C	ADG
40/10	8	–0.05x	10	0.29	15	0.47	20	1.13	Cool	0.61x
	26	2.27y	26	1.66	26	1.43	26	1.10	Warm	2.58y
48/18	8	–0.04x	10	0.33	15	0.44	20	1.14	Cool	0.62x
	26	3.24z	26	1.93	26	1.56	26	0.89	Warm	3.07z
Pooled SEM		0.07		0.04		0.06		0.09		0.05
Main effect means ^c										
Diet	40/10	1.15		1.03y		1.05		1.12		2.11
	48/18	1.64		1.19z		1.10		1.01		1.86
Temp	Cool	–0.02		0.34Y		0.51Y		1.14		0.76
	Warm	2.81		1.87Z		1.64Z		0.99		3.20
Analysis of variance, Pr > F										
Diet (D)		0.01		0.04		0.48		0.33		0.016
Temperature (T)		<0.001		<0.001		0.01		0.21		<0.001
D × T		0.01		0.11		0.42		0.29		0.05

^aWeight gained/100 g of initial fish in that temperature interval per day.
^bDiets are designated % protein/% lipid on an as-fed basis.
^cMain effect (least squares) means in each column with different letters (lowercase = diet differences; uppercase = temperature differences) are different (*P* < 0.10) as determined by the Tukey–Kramer method for pair-wise comparisons (Prins et al. 2003). In the case of significant interaction, diet × temperature treatment means with different letters are significantly different.

TABLE 4. *Feed efficiency (FE) of juvenile sunshine bass (154 g) fed two commercial diets differing in protein and energy ratio and reared for 14 wk at temperatures simulating winter–spring in the Southeastern USA. Cool refers to tanks in which temperatures were warmed from 8 to 20 C; warm tanks were held at 26 C. Values in each column are mean response of duplicate tanks of fish in each diet × temperature treatment combination for 2-wk (8 vs. 26 C), 4-wk (10, 15, or 20 vs. 26 C) or 14-wk (cool vs. warm) intervals.*

Diet ^b	C	FE ^a	C	FE	C	FE	C	FE	C	FE
40/10	8	−0.15	10	0.56	15	0.43x	20	0.56	Cool	0.50
	26	0.69	26	0.71	26	0.63y	26	0.62	Warm	0.65
48/18	8	−0.13	10	0.67	15	0.44x	20	0.68	Cool	0.58
	26	1.00	26	0.91	26	0.89z	26	0.75	Warm	0.86
Pooled SEM		0.08		0.07		0.02		0.05		0.04
Main effect means ^c										
Diet	40/10	0.27		0.63		0.53		0.59y		0.58y
	48/18	0.44		0.79		0.67		0.71z		0.72z
Temp	Cool	−0.14Y		0.62Y		0.44		0.62		0.54Y
	Warm	0.84Z		0.81Z		0.76		0.68		0.76Z
Analysis of variance, Pr > F										
Diet (D)		0.49		0.10		0.01		0.10		0.04
Temperature (T)		0.02		0.07		<0.001		0.32		0.02
D × T		0.55		0.60		0.01		0.95		0.21

^aWeight gained/amount of feed consumed (dry-weight basis) in that temperature interval.

^bDiets are designated % protein/% lipid on an as-fed basis.

^cMain effect (least squares) means in each column with different letters (lowercase = diet differences; uppercase = temperature differences) are different ($P < 0.10$) as determined by the Tukey–Kramer method for pair-wise comparisons (Prins et al. 2003). In the case of significant interaction, diet × temperature treatment means with different letters are significantly different.

In cool water, fish fed the 40/10 diet had larger livers than fish fed the 48/18 diet. IPF ratio ranged from 6.3 to 8.3% of body weight and differed with respect to diet (Table 5). Fish fed the 48/18 diet accumulated slightly less than 2% points more in IPF than fish fed the 40/10 diet (a gain of about 33%), but there were no differences in IPF ratio with respect to culture temperature. Fillet yield, as measured by MR, ranged from 40 to 42% of body weight and did not differ between diets (Table 5). However, fillet yield from fish held at 26 C was marginally greater ($P = 0.104$) than that of fish held in cool water. Whole-body lipid status differed between culture temperatures depending on the diet fed (Table 5). Fish fed the 48/18 diet exhibited greater whole-body lipid (2.7–5.3% points greater) and a higher percent increase (63–86%) from initial lipid content than fish fed the 40/10 diet regardless of temperature. However, whole-body lipid or percent change from initial content was not different in fish fed the 40/10 diet at the two temperature regimes. Mean PRE ranged from 22.9 to 30.6% and was

unaffected by diet (Table 5). However, warm water (26 C) fish gained more protein per gram of protein fed than cool water fish. Mean ERE was independently and significantly influenced by diet and culture temperature (Table 5). Fish fed the 48/18 diet retained more energy per calorie fed than those fed the 40/10 diet regardless of culture temperature and warm water fish retained more energy per calorie fed than cool water fish.

Summer Culture

Feeding and Growth. At 29 C, fish gained 38–60% of their initial weight in 4 wk and final average weights ranged from 141 to 164 g/fish (Table 6). At 32 C, fish gained 50–67% of their initial weight in 4 wk and final average weights ranged from 166 to 190 g/fish (Table 7). In the 29 C trial, 2 of 240 fish were lost due to escape from their tanks. In the 32 C trial, 7 of 240 died with no discernable correlation to diet protein or lipid level; 5 of the 12 tanks lost one fish out of 20 and one tank lost two fish. Fish fed diets containing 40% protein were heavier

TABLE 5. *Body compositional indices and protein and energy retention in juvenile sunshine bass (154 g initial weight) fed two commercial diets differing in protein and energy ratio and reared for 14 wk at temperatures simulating winter-spring in the Southeastern USA. Cool refers to those tanks in which water temperature was manipulated from 8 to 20 C; warm refers to those tanks held at 26 C. Values in each column are mean response of five fish/tank (N = 2) in each treatment combination.*

Diet ^a	Temp.	HSI ^b	IPF ratio ^c	Muscle ratio ^d	Whole-body lipid	Whole-body lipid change ^e	PRE ^f	ERE ^g
40/10	Cool	2.70z	6.40	40.1	12.0x	33.1x	23.5	27.9
	Warm	1.80x	6.30	42.3	11.3x	26.2x	30.2	32.1
48/18	Cool	2.20y	8.00	40.8	14.7y	63.4y	22.9	36.1
	Warm	1.70x	8.30	41.9	16.7z	86.0z	30.6	47.3
Pooled SEM		0.08	0.52	1.0	0.5	5.2	1.2	2.4
Main effect means ^h								
Diet	40/10	2.25	6.34y	41.2	11.7	29.6	26.9	30.0y
	48/18	1.96	8.12z	41.4	15.7	74.7	26.7	41.7z
Temperature	Cool	2.45	7.19	40.5Y	13.3	48.3	23.2Y	32.0Y
	Warm	1.77	7.26	42.1Z	14.0	56.1	30.4Z	39.7Z
Analysis of variance, Pr > F								
Diet (D)		0.002	0.002	0.89	0.001	<0.001	0.91	0.02
Temperature (T)		<0.001	0.89	0.10	0.14	0.14	0.01	0.05
D × T		0.06	0.65	0.56	0.01	0.01	0.71	0.25

^aDiets are designated % protein/% lipid on an as-fed basis.

^bHepatosomatic index (HSI) = liver mass × 100/fish mass.

^cIntraperitoneal fat (IPF) ratio = intraperitoneal fat mass × 100/fish mass.

^dMuscle ratio (MR) = fillet yield with ribs × 100/fish mass.

^e% Change from initial whole-body lipid content (9%).

^fProtein retention efficiency (PRE) = protein gain × 100/protein fed.

^gEnergy retention efficiency (ERE) = energy gain × 100/energy fed.

^hMain effect (least squares) means in each column with different letters (lowercase = diet differences; uppercase = temperature differences) are different ($P < 0.10$) as determined by the Tukey–Kramer method for pair-wise comparisons (Prins et al. 2003). In the case of significant interaction, diet × temperature treatment means with different letters are significantly different.

($P < 0.10$) than those fed diets containing 35% protein at both 29 and 32 C. The influence of dietary lipid level on fish weight, however, depended on both culture temperature and dietary protein level. At 29 C, dietary lipid level did not affect final fish weight (Table 6). At 32 C, 15% dietary lipid resulted in heavier fish than 10% dietary lipid when fish were fed the 35% protein diets. However, at 40% dietary protein, dietary lipid level did not affect final fish weight at 32 C (Table 7). ADGs of fish fed the 40% protein diets were greater than those of fish fed the 35% diets at either 29 or 32 C, regardless of dietary lipid levels. Feed intake was affected by both culture temperature and dietary protein level. At 29 C, fish consumed more 40% protein diet, regardless of dietary lipid level (Table 6), whereas at 32 C feed intake did not differ among diets (Table 7). FE was positively and independently related to

both protein and lipid levels in the diet when fish were held at either 29 or 32 C.

Body Compositional Indices and Retention Efficiencies. Liver size (HSI) ranged from 2 to 4% of body weight at the end of the two summer culture trials (29 and 32 C). Larger livers were found in fish fed diets containing the lower protein (35%) or lower lipid (10%) levels at either 29 C (Table 6) or 32 C (Table 7). Body fat (IPF) ranged from 5.7 to 7% of fish weight; however, accumulation of IPF depended on temperature as well as dietary protein and lipid level. At 29 C, IPF was not influenced by diet (Table 6), whereas at 32 C slightly more body fat was found in fish fed the higher protein (40%) or lipid (15%) level (Table 7). MR depended on culture temperature and dietary protein, but not on lipid level of

TABLE 6. Growth, body compositional indices, and protein and energy retention in juvenile sunshine bass (103 g initial weight) fed four commercial diets differing in protein and lipid levels for 4 wk at 29 C. Values in each column are mean response of triplicate tanks in each treatment combination. Final average fish weights are based on individual weights of 10 fish/tank and body indices are based on five fish/tank (N = 3) in each treatment combination.

Diet rotein ^a	Diet lipid ^a	Final fish weight (g)	Daily gain ^b	Feed Intake ^c	FE ^d	HSI ^e	IPF ratio ^f	Muscle ratio ^g	PRE ^h	ERE ⁱ
35	10	143.8	1.49	2.23	0.65	3.18	6.07	39.5	29.2	30.1
	15	140.9	1.36	1.91	0.69	2.37	5.87	38.5	30.5	31.6
40	10	164.0	1.76	2.41	0.74	2.50	5.67	40.3	27.5	36.1
	15	163.9	2.12	2.39	0.87	1.99	6.17	40.3	31.7	31.3
Pooled SEM		7.9	0.20	0.14	0.05	0.29	0.60	1.3	3.3	4.5
Main effect means ^j										
Protein	35	140.0y	1.42y	2.07y	0.67y	2.89z	6.01	39.0y	29.9	30.8
	40	158.2z	1.94z	2.40z	0.80z	2.18y	5.95	40.3z	29.6	33.7
Lipid	10	154.3	1.63	2.32	0.69Y	2.76Z	5.91	39.9	28.3	33.1
	15	143.9	1.74	2.15	0.78Z	2.30Y	6.05	39.4	31.1	31.4
Analysis of variance, Pr > F										
Protein (P)		0.03	0.01	0.02	0.02	<0.001	0.83	0.03	0.92	0.48
Lipid (L)		0.19	0.50	0.18	0.08	0.008	0.62	0.38	0.36	0.68
P × L		0.17	0.16	0.23	0.34	0.23	0.25	0.36	0.62	0.45

^aDiets are designated % protein and % lipid on an as-fed basis.

^bWeight gained/100 g of initial fish per day.

^cAmount of feed consumed/100 g of initial fish per day.

^dFeed efficiency (FE) = g gained/g dry feed consumed.

^eHepatosomatic index (HSI) = liver mass × 100/fish mass.

^fIntraperitoneal fat (IPF) ratio = intraperitoneal fat mass × 100/fish mass.

^gMuscle ratio (MR) = fillet yield with ribs × 100/fish mass.

^hProtein retention efficiency (PRE) = protein gain × 100/protein fed.

ⁱEnergy retention efficiency (ERE) = energy gain × 100/energy fed.

^jMain effect (least squares) means in each column with different letters (lowercase = protein differences; uppercase = lipid differences) are different ($P < 0.10$) as determined by the Tukey–Kramer method for pair-wise comparisons (Prins et al. 2003).

the diet. At 29 C, fish fed the 40% protein diets exhibited greater MR than those fed the 35% diets (Table 6), whereas at 32 C, MR averaged 43.5% and appeared unaffected by diet (Table 7). PRE was influenced by culture temperature as well as dietary protein and lipid level. At 29 C, PRE averaged 34% and was unaffected by diet (Table 6). At 32 C, fish retained more protein when fed diets containing 40%, as opposed to 35%, protein; however, lipid level only influenced protein retention at the lower dietary protein level (Table 7). At 35% dietary protein, fish fed the diet containing 15% fat retained more protein than those fed the 35/10 diet at 32 C. ERE was influenced by culture temperature and lipid level, but not protein level, in the diet. At 29 C, ERE averaged 31% and was not different among diets (Table 6). At 32 C, ERE was greater in fish

fed the higher lipid level but was unaffected by dietary protein level (Table 7).

Discussion

The response data from the winter culture trial suggest that the lower limit of feeding activity in hybrid striped bass is around 8 C or approximately 6 C above the lower lethal limit of sunshine bass fed prepared diets (Kelly and Kohler 1999). Daily rates of intake and gain as well as temperature-period feed efficiencies exhibited trends consistent with known effects of culture temperature and fish size. Intake, gain, and FE increased with increasing water temperature in the cool water treatment (8–20 C) but decreased over time in the warm water treatment (26 C) with the latter trend reflecting increasing fish size at the constant optimum culture temperature.

TABLE 7. Growth, body compositional indices, and protein and energy retention efficiencies of juvenile sunshine bass (113 g initial weight) fed four commercial diets differing in protein and lipid levels for 4 wk at 32 C. Values in each column are mean response of triplicate tanks in each treatment combination. Final average fish weights are based on individual weights of 10 fish/tank and body indices are based on five fish/ tank (N = 3) in each treatment combination.

Diet protein ^a	Diet lipid ^a	Final fish weight (g)	Daily gain ^b	Feed Intake ^c	FE ^d	HSI ^e	IPF ratio ^f	Muscle ratio ^g	PRE ^h	ERE ⁱ
35	10	166.2	1.81	2.72	0.68	4.08	5.94	43.5	25.0y	32.4
	15	179.9	1.96	2.65	0.74	3.68	6.64	43.6	31.5z	38.0
40	10	190.0	2.23	2.82	0.77	3.07	6.48	43.4	32.8z	35.2
	15	184.2	2.39	2.87	0.85	2.04	6.93	44.5	31.4z	41.0
Pooled SEM		6.8	0.22	0.18	0.03	0.33	0.36	1.3	0.65	1.6
Main effect means ^j										
Protein	35	173.1y	1.88y	2.69	0.71y	3.88z	6.29y	43.5	28.3	35.2
	40	187.1z	2.31z	2.84	0.81z	2.56y	6.71z	44.0	32.1	38.1
Lipid	10	178.1	2.02	2.77	0.73Y	3.58Z	6.21Y	43.5	28.9	33.8Y
	15	182.1	2.17	2.76	0.79Z	2.86Y	6.79Z	44.0	31.5	39.5Z
Analysis of variance, Pr > F										
Protein (P)		0.06	0.09	0.42	0.01	<0.001	0.07	0.61	0.001	0.12
Lipid (L)		0.56	0.51	0.94	0.05	0.001	0.02	0.52	0.01	0.008
P × L		0.17	1.00	0.74	0.64	0.11	0.57	0.58	0.001	0.94

^aDiets are designated % protein and % lipid on an as-fed basis.

^bWeight gained/100 g of initial fish per day.

^cAmount of feed consumed/100 g of initial fish per day.

^dFeed efficiency (FE) = g gained/g dry feed consumed.

^eHepatosomatic index (HSI) = liver mass × 100/fish mass.

^fIntraperitoneal fat (IPF) ratio = intraperitoneal fat mass × 100/fish mass.

^gMuscle ratio (MR) = fillet yield with ribs × 100/fish mass.

^hProtein retention efficiency (PRE) = protein gain × 100/protein fed.

ⁱEnergy retention efficiency (ERE) = energy gain × 100/energy fed.

^jMain effect (least squares) means in each column with different letters (lowercase = protein differences; uppercase = lipid differences) are different ($P < 0.10$) as determined by the Tukey–Kramer method for pair-wise comparisons (Prins et al. 2003).

Buentello et al. (2000) also found increasing feed intake in channel catfish when culture temperatures mimicked winter–spring warming from 16 to 32 C. In that study, FE and weight gain also increased with increasing culture temperature up to 24–26 C and 28 C, respectively and then declined as temperature increased to approximately 32 C. In the current study, daily intake in the warm water group was greater than that of the cool water group when temperatures ranged from 8 to 15 C in the cool water group. On the other hand, intake in the warm water group was less than that of the cool water group when temperatures ranged from 15 to 20 C in the cool water group. This is a consequence of fish achieving greater mean weight (463 g) in the warm water group than fish in the cool water group (185 g) after 10 wk of growth, as well as the larger fish consuming

less (1.5 vs. 1.8%, respectively) on a percent body weight basis.

Because both protein retention and MR were unaffected by diet, the compositional data reveal that the few instances of greater gains and efficiencies observed at cooler temperatures in fish fed the 48/18 diet, as opposed to the 40/10 diet, were probably a result of greater deposition of dietary energy in the form of intraperitoneal, liver, and whole-body lipid and/or glycogen stores, rather than greater protein deposition. Whole-body lipid, which averaged 9% at the start of the trial, increased to 12% in fish fed the 40/10 diet and 16% in fish fed the 48/18 diet regardless of temperature. About half (1.8%) of the increase in whole-body lipid in fish fed the 48/18 diet can be accounted for by the increase in IPF. Moreover, fat accretion more than doubled (>18% total

body lipid) in 7 of 40 fish sampled from the 48/18 dietary treatment.

In general, the attenuated responses combined with the lower MRs observed in fish held in cool water regardless of diet, corroborate results of recent energetic studies in another carnivorous fish, red drum, *Sciaenops ocellatus*. Fontaine et al. (2007) suggested that metabolic capacity in fish tends to limit performance at lower temperatures rather than feed energy density. In spite of the stark difference in initial fish size between the latter (ca. 2 g initial weight) and the current (ca. 100 g initial weight) study, both studies found that growth increased with temperature and at a greater rate for fish fed high-energy diets as compared to low-energy diets and no differences in growth rate due to diet energy were observed below ambient. Indeed, both routine metabolic rate (RMR) and metabolic scope (MS), which measures capacity for physiological performance, were significantly depressed in red drum and were not altered by diet energy below 25 C (Fontaine et al. 2007).

Another application of the energetic explanation is that not only were livers larger in fish held in cool water regardless of diet, but also fish fed the 40/10 diet had larger livers than those fed the 48/18 diet in cool water. The latter observation may seem counterintuitive, but the 40/10 diet contained more NFE carbohydrate (36%) than the 48/18 diet (22%). Stone and Sidell (1981) found a relatively larger increase in pentose phosphate cycle products from radiolabeled glucose in cold acclimated (5–15 C) striped bass but a relatively larger increase in labeled palmitate oxidation in warm acclimated (25 C) fish and concluded that carbohydrate was the metabolically preferred substrate at cold temperature, whereas lipid was preferred at warm temperatures. If this were true, then fish held in cool water should have gained more fat than those held in warm water and even more so for those fed the 48/18, i.e., higher fat, diet. As noted in Rawles et al. (2008), it is more likely that the *in vitro* trends observed by Stone and Sidell (1981) in cold temperatures reflect hepatic, rather than whole-body, changes in substrate use and more

particularly an increase in carbohydrate storage capacity through enhanced production of reducing equivalents in the pentose phosphate cycle rather than carbohydrate oxidation. Although liver glycogen was not measured in the current study, high deposition of dietary carbohydrate in the liver of *Morone* spp. is well characterized (Hutchins et al. 1998; Rawles et al. 1998; Burr et al. 2006). Hence, inefficient energy use, i.e., increased storage, of dietary carbohydrate in cool water most likely accounts for the larger livers observed in fish fed the 40/10 diet.

Because the response to diet was, in most cases, not different below 20 C, the results do not support the strategy of feeding a high density, 48/18 commercial diet to hybrid striped bass in deep winter. Above 15 C, however, hybrid striped bass consumed less of the 48/18 diet for similar gains and better FE than those fed the 40/10 diet and both lipid and energy retention increased in the former fish. Similarly, Fontaine et al. (2007; Tables 3 and 4) found increased FE, growth rate, and nutrient and energy retention in red drum fed a nutrient-dense diet at 25 C. Interestingly, the difference in protein and lipid content between the 40/10 and 48/18 diets of the current study was less severe than that in the low- and high-energy diets employed by Fontaine et al. (2007). This suggests why red drum fed the low-energy diet had virtually no IPF when compared to those fed the high-energy diet; however, we observed minor (2%), although statistically different, increases in IPF in hybrid striped bass fed a high energy diet. The current results suggest that there is merit to feeding a nutrient-dense diet to hybrid striped bass in spring when temperatures are consistently above 15 C. Peres and Oliva-Teles (1999) also found improved performance and nutrient retention efficiencies in European sea bass fed nutrient-dense diets between 18 and 25 C. Nevertheless, a cost–benefit analysis is necessary to determine if the increased expense of the 48/18 diet is offset by the enhanced production.

With regard to the summer temperature trials, it is noteworthy that no interaction between dietary protein and lipid level was observed in the response of hybrid striped bass to 29 C,

whereas significant interaction was found at 32 C. Growth performance and compositional indices were highly influenced by dietary protein at 29 C of the current study. Burr et al. (2006) also found that weight gains and FE improved while liver size (HSI) decreased with increasing dietary protein level in hybrid striped bass. Additionally, both the latter and current study found dietary lipid level did not particularly influence hybrid striped bass growth, body compositional indices, or nutrient retentions at 25 and 29 C. At 29 C, only FE and liver size of hybrid striped bass were affected by dietary lipid level in the current study, whereas only feed intake and whole-body protein were marginally affected by dietary lipid level in Burr et al. (2006). On the other hand, at 32 C protein retention in fish fed the 35% protein diets increased when dietary fat was increased to 15%; this suggests protein sparing by lipid at the marginal protein level when energetic needs are greater at the higher temperature regime.

Interestingly, when responses to diet nutrient density across the three trials are qualitatively compared, lipid stores appeared greater in similar-sized fish held at the cooler temperatures. For example, hybrid striped bass held at 8–20 C contained 7.2% body fat (IPF) on average, whereas those held at 29 or 32 C contained less than 6.5% body fat on average and less than 6% in many cases. These data tend to support the findings of Santulli et al. (1993) that dietary lipid assimilation decreases in temperate basses as water temperature increases as well as the idea that increasing metabolic rate at higher temperature would leave less dietary energy available for deposition as described by Jobling (1995). On the other hand, gain as well as protein and energy retentions at 29 C appeared somewhat lower than those observed at 32 C and this would contradict results from Keembiyehetty and Wilson (1998) who found growth and nutrient utilization were greater at 27 C as compared to 32 C. Interestingly, Keembiyehetty and Wilson (1998) likewise observed greater feed intake at the higher temperature. Keembiyehetty and Wilson (1998) attributed the former observation to increased energy requirements for maintenance and activity in

fish held at the higher temperature, which is corroborated by the previously noted energetic studies.

We can only speculate as to the reasons for the putative discrepancies in response to high temperature between our study, previous work with sunshine bass, and fish energetics theory. First, it may be important that Keembiyehetty and Wilson (1998) found no differences in the pattern of nutrient use with respect to diet nutrient density and high culture temperature; however, we found significant differences due to both protein and lipid levels in the diet. Secondly, because our trials were conducted serially rather than concurrently, statistical comparison between our high-temperature trials is precluded and other fish or culture system factors may have come into play. Thirdly, fish size was radically different between studies: Keembiyehetty and Wilson (1998) fed fish that began at less than 4 g each, whereas we fed fish that were 20-fold greater in size (100–113 g). Additionally, Keembiyehetty and Wilson (1998) fed cold-pelleted diets containing semi-purified protein, carbohydrate, and fiber sources, whereas we fed extruded, commercial diets containing typical feedstuffs that can tremendously alter nutrient digestion and assimilation efficiencies. Finally, the amount of time allowed for response in each trial was significantly different. Keembiyehetty and Wilson (1998) fed fish for 8 wk, whereas we fed fish for 4 wk in the warm water trials. As noted below, response to diet \times temperature interaction can take significantly longer to manifest than either 4 or 8 wk (Person-Le Ruyet et al. 2004), although the shorter periods of time at temperature will reflect pond production conditions more closely.

The literature suggests that fish lose appetite and conversion efficiencies decrease at the extremes of their temperature tolerance range (Kestemont and Baras 2001). Consistent with energetics theory (Brett and Groves 1979), Fontaine et al. (2007) speculated that growth in red drum would decrease at temperatures higher than 29 C as the upper lethal limit was approached. Claireaux and Lagardère (1999) measured temperature effects on the

energetics of another moronid, European sea bass, *Dicentrarchus labrax*, and observed that a temperature rise from 10 to 20 C resulted in a 2.5-fold increase in standard metabolic rate (SMR) and a 6-fold increase in active metabolic rate (AMR); however, SMR leveled off from 20 to 25 C and no further increase was seen at the same time that AMR slightly decreased. This suggests that the difference between AMR and SMR, i.e., the capacity for physiological performance (MS), decreases at 25 C or above. Indeed, up to Day 72, Person-Le Ruyet et al. (2004) saw no difference in gains between 25 and 29 C in European sea bass, however gain decreased from Days 72 to 84 at 29 C. Nevertheless, we observed remarkable adaptation and growth response to dietary protein and lipid at 32 C in advanced juvenile hybrid striped bass during the 30-d trial. Hybrid striped bass may have a wider tolerance for changes in environmental temperature than most fish, including red drum or European sea bass. Woiwode and Adelman (1991) reported critical thermal maxima ranging from 28 to 40.5 C for sunshine bass, depending on acclimation temperature, and Beitinger et al. (2000) noted sunshine bass among a small number of taxa that "apparently establish the upper biokinetic limit for ectothermic vertebrates." The reasons for this adaptability to high temperature are unknown but may be related to an anticipatory seasonal metabolic compensation (Chipps et al. 2000) that potentially allows some *Morone* to tolerate transitory high temperatures in tidal estuaries.

In conclusion, the hypothesis that sunshine bass will perform better on a commercial diet of lower protein but higher lipid content during periods of extreme high temperature is not borne out by the current results. At 32 C, FE, body composition, and nutrient retentions were highly influenced by both dietary lipid and protein levels. Specifically, the higher protein level (44% on a dry-weight basis) was just as important as lipid in positively influencing those responses at 32 C. Therefore, while work remains to determine the effects of culture temperature and dietary nutrient density on stress and disease survivability, the current results suggest that judicious feeding of nutrient-dense,

commercial diets during spring or summer will improve the production efficiency of hybrid striped bass.

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